

***Trypanosoma cruzi* Infection in *Didelphis marsupialis* in Santa Catarina and Arvoredo Islands, Southern Brazil**

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Between 1984 and 1993 the prevalence of the Trypanosoma cruzi infection in opossums (Didelphis marsupialis) was studied in Santa Catarina and Arvoredo Islands, State of Santa Catarina, Brazil. The association of the triatomine bug Panstrongylus megistus with opossums nests and the infection rate of these triatomines by T. cruzi was also studied. Thirteen different locations were studied in Santa Catarina Island (SCI), in which 137 D. marsupialis were collected. Sixty two opossums were collected at the Arvoredo Island (AI), located 12 miles north from SCI. All captured animals were submitted to parasitological examinations that revealed the presence of T. cruzi in 21.9% of the opossums captured in SCI and 45.2% among opossums captured in the AI. The presence of P. megistus was detected in most of the D. marsupialis nests collected in the SCI, however, in the non-inhabited AI only eight triatomines were collected during the whole study. The presence of T. cruzi-infected D. marsupialis associated with P. megistus in human dwellings in the SCI, and the high infection rate of D. marsupialis by T. cruzi in the absence of a high vector density are discussed.

Key words: *Didelphis marsupialis* - *Trypanosoma cruzi* - *Panstrongylus megistus* - Chagas disease - human dwellings - Santa Catarina - Brazil

Trypanosoma cruzi, the etiological agent of Chagas disease, is a protozoan parasite that infects over 200 sylvatic or domestic mammalian species and subspecies from seven different orders in Central and South America, including man. Marsupials, mainly those from the genus *Didelphis*, have been cited as one of the most important reservoirs of *T. cruzi* in several Latin American countries (Barretto et al. 1964, Travi et al. 1994). Previous studies revealed distinct infection rates of *Didelphis* sp. by *T. cruzi* in different regions of Brazil such as 35.7% in Rio de Janeiro (Guimarães & Jansen 1943), 24% in the Amazon basin (Miles 1976), 20.6% in Mambuí, State of Goiás (Mello 1982) and 37.9% in Bambuí, State of Minas Gerais (Fernandes et al. 1991).

Due to its synantropic behaviour, frequently invading human dwellings in both rural and urban areas, opossums are commonly bitten by sylvatic and/or peridomestic triatomine species. Once infected by *T. cruzi* these marsupials usually present

high infection rates and long term parasitemia, acting as important links between the parasite sylvatic and domestic transmission cycles (Barretto et al. 1964, Zeledon et al. 1970).

Although the State of Santa Catarina is non-endemic for human Chagas disease, previous studies revealed that 84.5% and 66.6% of the *P. megistus* and *Rhodnius domesticus* were infected by *T. cruzi*, respectively (Schlemper Jr et al. 1985). Recently, adults and nymphs of *P. megistus* were found in several artificial ecotopes as well as in human dwellings in Santa Catarina Island (SCI), indicating the high potential of this species to invade and colonize human dwellings as observed in the State of São Paulo (Forattini et al. 1982, Steindel et al. 1994).

In this work we studied the prevalence of *T. cruzi* among *D. marsupialis* (= *D. aurita*) captured in two distinct islands in Santa Catarina as well as discuss the presence of this opossum associated with *P. megistus* in human dwellings as an important risk factor for the transmission of Chagas disease.

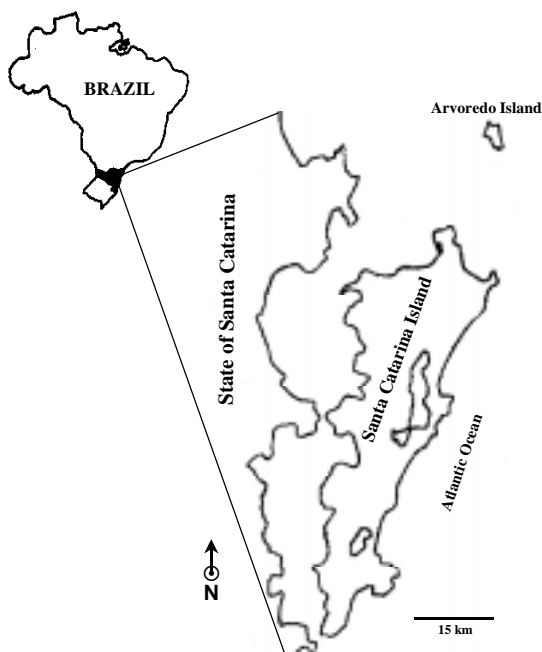
MATERIALS AND METHODS

Study site - SCI, also known as Florianópolis, has an area of 425 km² and is located in Santa Catarina, southern Brazil. Around 500,000 people live nowadays in this Island where only 15-20% of the original Atlantic forest remains intact. For

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Map showing the Arvoredo and Santa Catarina Islands localization off the State of Santa Catarina coast.

this study, SCI was divided in north, central and south regions, and 13 localities were studied. Arvoredo Island (AI), with a 7 km² area, is located 12 miles north from the SCI, mostly covered by a well-conserved forest. In the AI, a Brazilian navy base (Marinha do Brasil) has only a few men (3-5) which usually stay periods of 12-24 months in it (Figure).

Animal captures - Animals were collected in both islands from 1984 up to 1993, manually or with live-traps (20x20x60 cm) set up in sylvatic, peri-domestic or domestic environments as described by Fernandes et al. (1990). Animals captured by inhabitants were also included in this study. After sex and weight determination, each animal was subjected to parasitological examinations to detect *T. cruzi* infection. Both fresh and Giemsa-stained smears, hemoculture in LIT (liver infusion tryptose) medium as described by Luz et al. (1994) and xenodiagnosis performed with 15 nymphs of 4th/5th instar of *P. megistus* was used to detect *T. cruzi* infection.

Whenever possible, the scent glands were examined for the presence of *T. cruzi*. Other marsupial and rodent species were captured in both islands during this study. These animals were also examined for *T. cruzi* infection as described above.

Animals captured in the AI were either examined in the field or brought to the laboratory. Thirty-

five opossums captured in the AI were tagged and released for recapture after four to six months. For nest localization, six adult *D. marsupialis* were followed using the method described by Miles (1976). Briefly, this method uses a backpack attached to the opossum, which contains a line reel. The animal is released in the same capturing site and the line tracked to the end. Comparison of the percentage of infected animals in both islands was performed by the chi-square (χ^2) test.

Triatomine search - In both islands, triatomines were systematically searched in opossums nests in tree holes, rocks, palm trees and bromeliads in both sylvatic or peri-domiliar environment and also in houses and other human-made dwellings. In the AI, triatomines were also captured using light traps. All captured triatomines had their intestinal contents and feces examined for the presence of flagellates by fresh and Giemsa-stained smears. *T. cruzi* was isolated by sub-inoculation of positive feces in Swiss albino mice or by xenoculture as previously described (Bronfen et al. 1989).

RESULTS

A total of 199 *D. marsupialis* were collected in both islands, 137 (63 males, 70 females and 4 undetermined) at the SCI and 62 (27 males and 35 females) at the AI. The *T. cruzi* infection rate among these animals was 21.9% and 45.2%, respectively (Table).

At SCI, 13 out of 137 animals were positive by fresh blood examination (9.5%), 25 by xenodiagnosis (18.2%) and 21 by hemoculture (15.3%) in a total of 30 *T. cruzi* positive opossums. Among all *D. marsupialis* captured in SCI, mostly from Lagoa da Conceição, Trindade and Córrego Grande localities (Central region), 27.7% of them were captured in human dwellings (houses and annexes), 17.5% in the peridomicile (storage houses) and 45.2% in the sylvatic environment (Table). No *T. cruzi* infection in scent glands was detected among 71 animals examined in this island. *T. cruzi* infection was found in 12.4% of the opossums captured in human-related dwellings.

Two other marsupial species (*Lutreolina crassicaudata* and *Marmosa cinerea*) were captured in SCI. No *T. cruzi* infection was detected among the 34 captured animals (28 *L. crassicaudata* and six *M. cinerea*).

At the AI, six out of 62 opossums were positive for *T. cruzi* by fresh blood examination (9.7%), 28 by xenodiagnosis (45.2%) and 12 by hemoculture (19.3%). Among the 28 *T. cruzi*-positive *D. marsupialis*, two had parasites in the scent glands (7.1%), as determined by fresh and Giemsa-stained smears. All positive samples were isolated by culture in LIT medium. The number of infected

TABLE

Number of *Didelphis marsupialis* captured in Santa Catarina and Arvoredo Islands, the percentage of *Trypanosoma cruzi* infected animals and the number of captured and *T. cruzi* positive animals per ecotope

	Total number	<i>T. cruzi</i> infected	Capture site ^a		
			House	Annexes	Forest
Santa Catarina Island					
North region	10	50%	1	4 (3)	5 (2)
Central region	112	20.5%	37 (6)	20 (8)	55 (9)
South region	15	13.3%	-	-	15 (2)
Sub total	137	21.9% ^b	38 (6)	24 (11)	75 (13)
Arvoredo Island	62	45.2% ^b	-	-	62 (28)
Total	199		38 (6)	24 (11)	137 (41)

a: number of opossums captured (number of *T. cruzi* infected animals); b: significant (p<0.001)

opossums in the AI was significantly higher than in SCI (p<0.001).

From a total of 35 opossums examined, tagged and released in the same capturing site in the AI, six females were recaptured. One female, originally negative for *T. cruzi* infection by all methods, became infected within a six month period as revealed by xenodiagnosis and hemoculture indicating active transmission in the AI. Following the method used by Miles (1976), six opossums were monitored in the AI. Two animals were recovered and their nests, one in a tree hole and the other under a rock formation, were negative for triatomines. Two other line ends were found in rocks and two were disrupted. Neither the 12 rodents (*Oryzomys* sp.) captured in this island nor all six soldiers of the Brazilian navy living there were positive for *T. cruzi* infection.

A total of eight *P. megistus* (five female adults and three nymphs) were captured in the AI during the whole study. The five females were captured using light traps, while the three nymphs were found in an opossum nest in a tree hole. Six triatomines (three nymphs and three adults) revealed the presence of *T. cruzi* in their feces. Two others were collected by Navy soldiers and preserved in alcohol.

In contrast, the search for triatomines in SCI revealed the presence of *P. megistus* in the sylvatic environment, where 268 nymphs and six adults were collected, as well as in human dwellings where 305 nymphs and 24 adults were captured. The *T. cruzi* infection rate among these triatomines was 86.1% and 55.3%, respectively.

During this study, 38 *T. cruzi* strains were isolated from *D. marsupialis* and 28 strains were isolated from *P. megistus* collected in both SCI and

AI. These strains were already characterized by Steindel et al. (1993, 1995) by biological, biochemical and molecular methods.

Despite the existence of *T. rangeli* in Santa Catarina (Grisard et al. 1999), none of the animals examined during this study were infected by this parasite.

DISCUSSION

Opossums from the genus *Didelphis* have a wide distribution in South America, being frequently observed in close association with triatomines and human dwellings in both sylvatic and urban areas. Rodents and marsupials, are not only the most important blood source for several triatomine species, but also their major source of *T. cruzi* infection (Rocha e Silva et al. 1975). Studies on *T. cruzi* infection in *Didelphis* sp. in different regions of Brazil revealed infection rates varying from 20.6% to 37.9% (Guimarães & Jansen 1943, Miles 1976, Mello 1982, Fernandes et al. 1991). In the present work *T. cruzi* infection rates of 21.9% and 45.2% were found in *D. marsupialis* captured in the Santa Catarina and Arvoredo Islands, respectively.

Previous studies reported the association of *T. cruzi* infected triatomines (*P. megistus* and *R. domesticus*) with rodent and marsupial nests in wild areas of the SCI (Leal et al. 1961, Schlemper Jr et al. 1985). Also, the association of *P. megistus* with *D. marsupialis* in artificial ecotopes, including human-made dwellings in SCI, has been reported (Steindel et al. 1994). Similar report was made by Forattini et al. (1982) in the State of São Paulo.

Using the precipitin test to evaluate the food source of these triatomines, Steindel et al. (1994) have found that 80.6% of the 31 adult *P. megistus*

captured in artificial ecotopes fed on humans. Moreover, *T. cruzi* infection was detected in 55.3% of these triatomines.

In the present study, 62 out of 137 *D. marsupialis* (45.2%) were captured in human dwellings in the SCI. Natural *T. cruzi* infection was confirmed in 17 (27.4%) of these opossums.

The massive destruction of the original Atlantic forest in the SCI allied to the construction of houses close to the remaining forest made contact of humans with both opossums and *P. megistus* frequent. In contrast, AI is a federal reserve which have a well conserved Atlantic forest. Besides the five Brazilian Navy houses, no other human-made dwelling is present in this island where *T. cruzi* circulates among animals in a sylvatic environment. Moreover, opossums meat is still appreciated as an exotic food by native inhabitants of SCI.

The presence of *T. cruzi* infected triatomines and opossums in human dwellings in the SCI, as well as the detection of both human and opossum blood in *T. cruzi*-infected triatomines, indicates the risk of transmission of this parasite to humans. The same epidemiological situation was observed in the State of São Paulo when Litvoc et al. (1990) detected the presence of *P. megistus* in *D. azarae* nests and an infection rate of 47.8% of these opossums by *T. cruzi*.

A related behavior was observed by Telford Jr and Tonn (1982) in the upper llanos of Venezuela. Studying the *T. cruzi* dynamics in *D. marsupialis*, these authors observed a prevalence of 55.2% and a close relation of this animal with *R. prolixus* and human dwellings.

In contrast, an extensive serological survey carried out among 5,831 inhabitants of the SCI revealed a prevalence of infection of 0.034% (Carobrez et al. 1992). Thus, *T. cruzi* transmission in SCI occurs almost exclusively between *P. megistus* and *D. marsupialis* in the sylvatic environment. Moreover, SCI presents a low density of *P. megistus* found in artificial ecotopes, such as human-made dwellings (Steindel et al. 1994).

Despite the low prevalence in humans, the occurrence of naturally infected reservoirs and vectors in domestic environments at SCI does not rule out the possibility of finding human infection in this habitat.

A comparison of serological and parasitological tests to detect *T. cruzi* infection in 116 *D. albiventris* captured in Bambuí, State of Minas Gerais, revealed that 97.7% of the infected animals were positive in both tests (Fernandes et al. 1990). Having used fresh and Giemsa-stained smears, hemoculture and xenodiagnosis to detect *T. cruzi* infection in opossums during this study, 34 negative animals were kept in the laboratory and fol-

lowed during two months by parasitological and serological tests (indirect immunofluorescence). Since only one opossum was positive by either tests, we conclude that *T. cruzi* infection in opossums can be easily detected by using parasitological methods.

T. cruzi infection rate among *D. marsupialis* captured in the AI, which is geographically isolated and has a well conserved forest, was 45.2%. In contrast with the high *T. cruzi* infection rate among opossums, triatomines are scarce. *P. megistus* was the only species captured in this island and all six triatomines examined were positive for *T. cruzi*. Moreover, opossum blood was detected in all three nymphs submitted to precipitin tests.

Two out of 28 (7.1%) opossums also presented *T. cruzi* in their scent glands. Natural *T. cruzi* infection in *D. albiventris* and *D. marsupialis* scent glands has been demonstrated by Fernandes et al. (1989) and by Naiff et al. (1987) which have detected one positive gland out of 20 animals examined, and in one out of 90, respectively. Our results are in agreement with these previous reports, confirming the low occurrence of naturally *T. cruzi*-positive scent glands in *D. marsupialis*. Moreover, we cannot infer that this possible transmission mechanism can be responsible for the high prevalence of *T. cruzi* in opossums of the AI. Other possibilities of vertical transmission such as milk feeding were studied and discarded by Telford Jr and Tonn (1982) and by Deane et al. (1986).

Characterization of 68 *T. cruzi* strains by biological, biochemical and molecular methods showed that strains from AI produce sub-patent parasitemia in Swiss mice and a high homogeneity of isoenzyme and randomly amplified polymorphic DNA profiles. Based on the same markers, strains isolated in SCI revealed a higher heterogeneity than that observed among strains isolated in the AI (Steindel et al. 1995).

These results can be explained by the geographical isolation of the AI, where *T. cruzi* strains circulate almost exclusively among opossums. On the other hand, in the SCI *T. cruzi* have been isolated from triatomines and a wide variety of mammals, rodents and marsupials which may explain the higher heterogeneity observed.

The presence of *T. cruzi* in the AI opossums scent glands may suggest a high adaptation of some parasite strains and the opossum. Deane et al. (1984, 1986) observed that only a few *T. cruzi* strains were able to infect the scent glands under controlled conditions.

Due to the opossums omnivorous habits, another way of infection considered was the ingestion of *T. cruzi*-infected rodents. All 12 *Oryzomys*

sp. captured and submitted to parasitological tests were negative for *T. cruzi* infection. We have not discarded this possibility, however, it appears to be infrequent.

Experimental infection of newborn *D. marsupialis* with *T. cruzi* strains from AI and SCI showed a long-term blood parasitemia. The presence of *T. cruzi* was observed in 50% of the scent glands of these animals after a two to three months period only in opossums experimentally infected with strains isolated from AI (M Steindel, unpublished data).

Infection of the opossum scent glands suggests a high degree of host-parasite adaptation of some *T. cruzi* strains. Trypanosomes derived from opossums scent glands proved to be infective for mice and opossums under experimental conditions (Deane et al. 1986, Steindel et al. 1988).

Another hypothesis that may explain the high *T. cruzi* prevalence among opossums in the AI that must be considered is the ingestion of *T. cruzi*-infected triatomines. The known insectivorous habits of these animals have already been demonstrated (Zeledon 1974) and must be considered as a possible *T. cruzi* infection source to the opossums in the AI. The low number of triatomines found in the AI did not explain the high prevalence of *T. cruzi* among opossums. However, more studies must be performed in order to better evaluate the triatomine density in this island. The existence of an alternative or unusual transmission mechanism of *T. cruzi* between *D. marsupialis* in the AI cannot be neglected.

Although Santa Catarina is not an endemic area for human Chagas disease, the presence of *D. marsupialis* infected with *T. cruzi* in human dwellings in the SCI must be considered as an important risk factor for Chagas disease. Moreover, serving as blood and *T. cruzi* infection source to *P. megistus*, these opossums are acting as links between the domestic and sylvatic transmission cycles.

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